Mechanism of Microbial Metal Reduction

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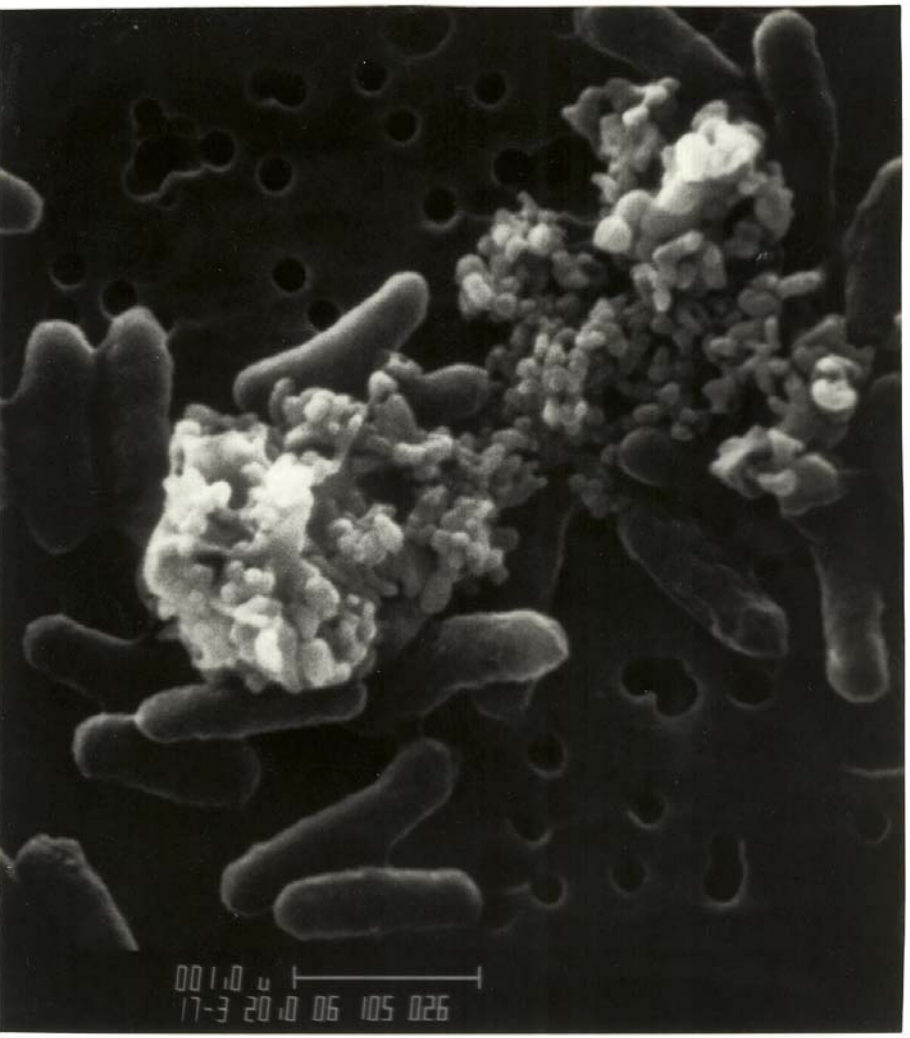
DOE Biogeochemistry Grand Challenge with PNNL:

Fe: **David Bates, Justin Burns, Christine Fennessey**
U: **Jason Dale**
Tc: **Amanda Payne**

ERSP collaboration with Andy Neal at SREL:

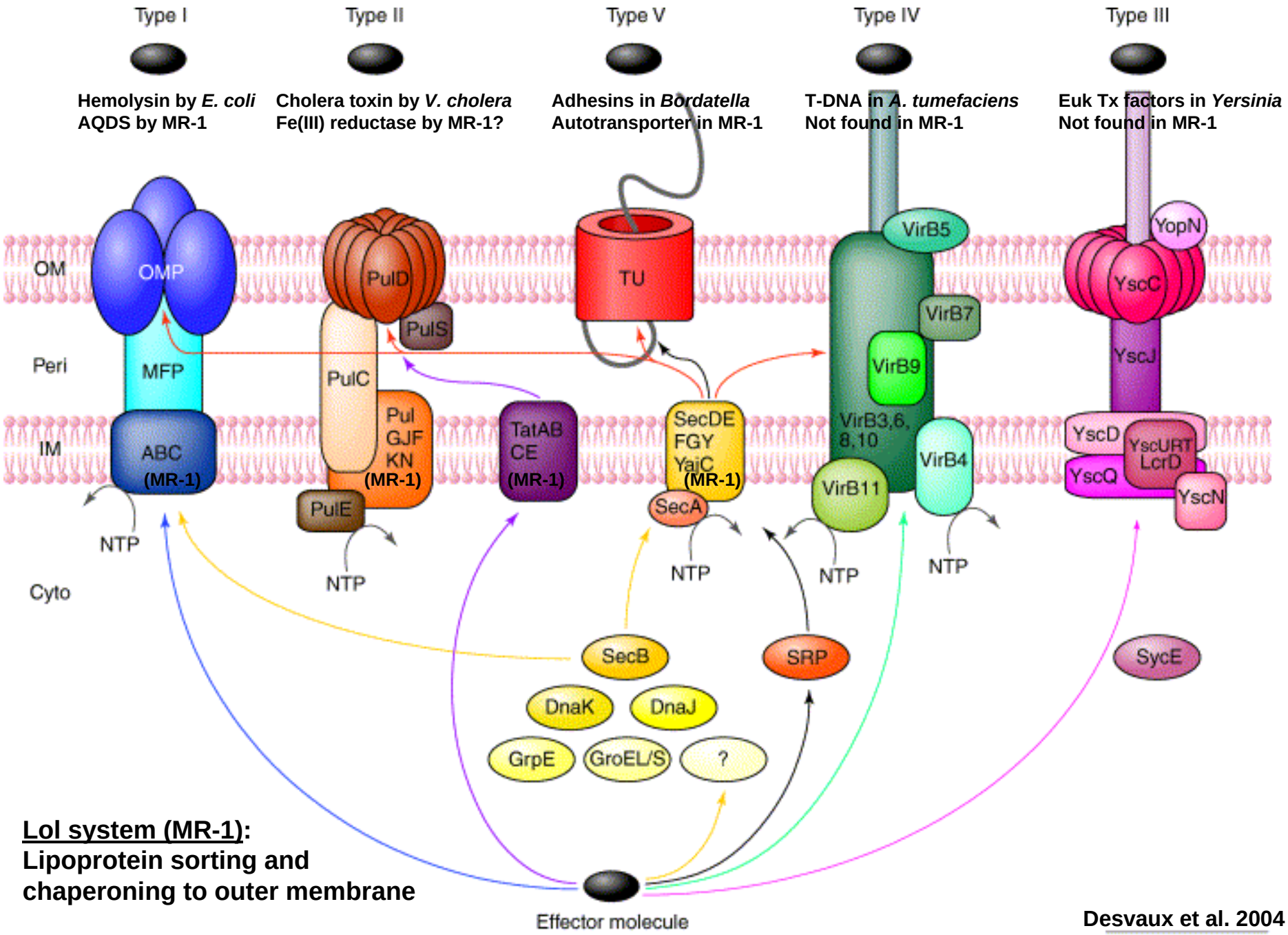
Cryo HR-SEM and laser doppler velocimetry

Physiological problem associated with respiration on solid electron acceptors



Potential solutions:

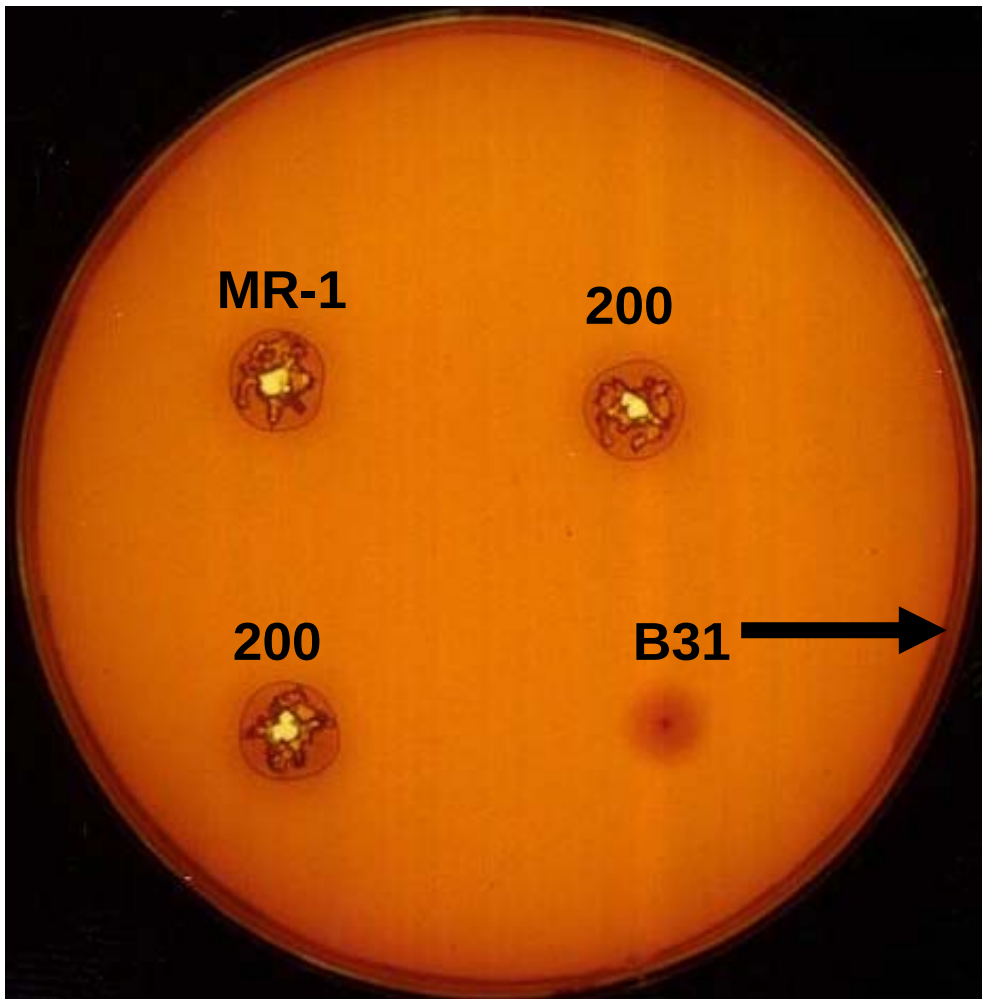
1. **Secrete metal reductase out to OM**
2. **Solubilize and transport metal in to IM**
3. **Exogenous electron shuttles**
4. **Endogenous electron shuttles**



Lol system (MR-1):
Lipoprotein sorting and chaperoning to outer membrane

Desvaux et al. 2004
TRENDS in Microbiology

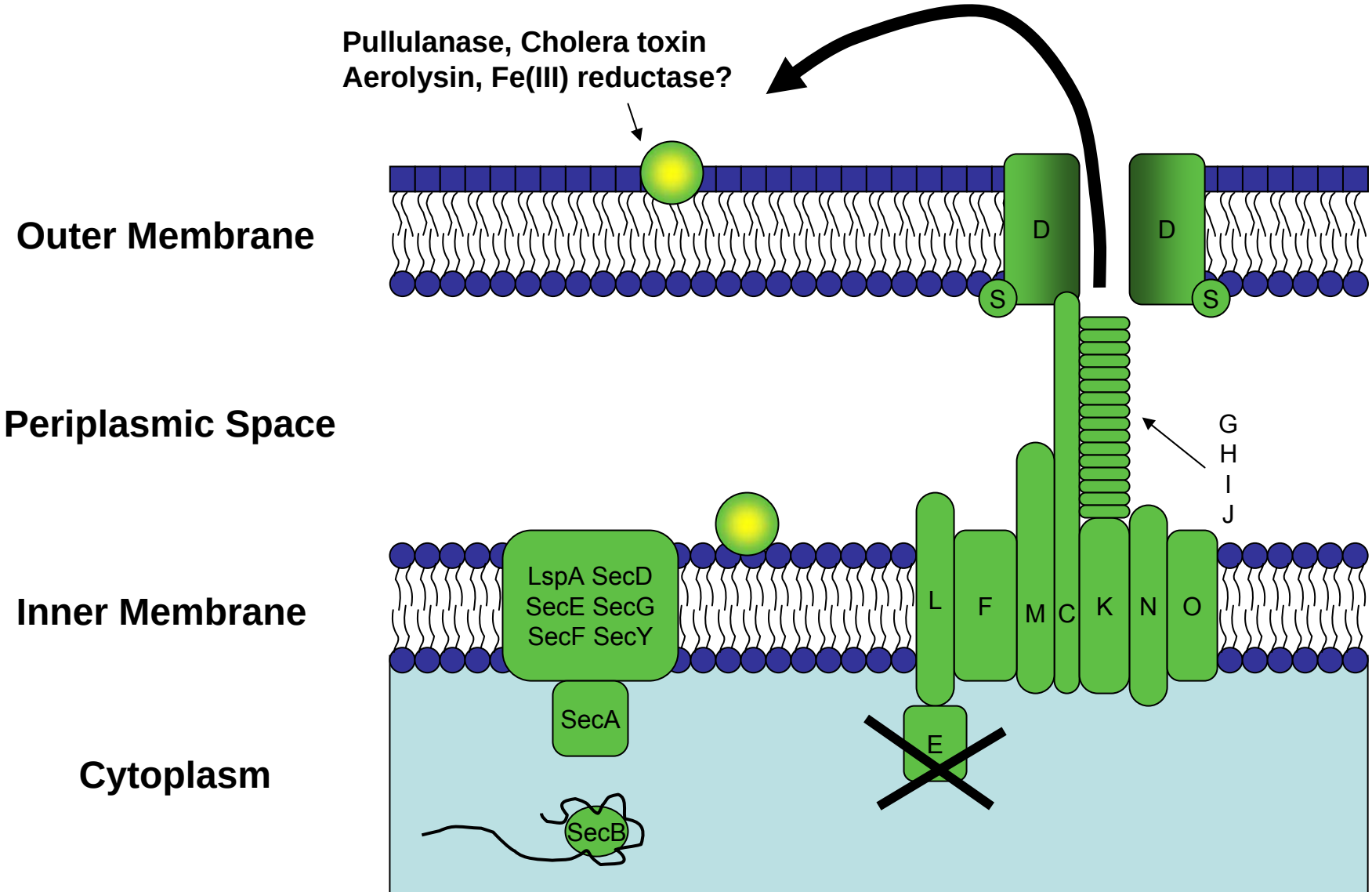
AGR mutant phenotype - Fenton reaction



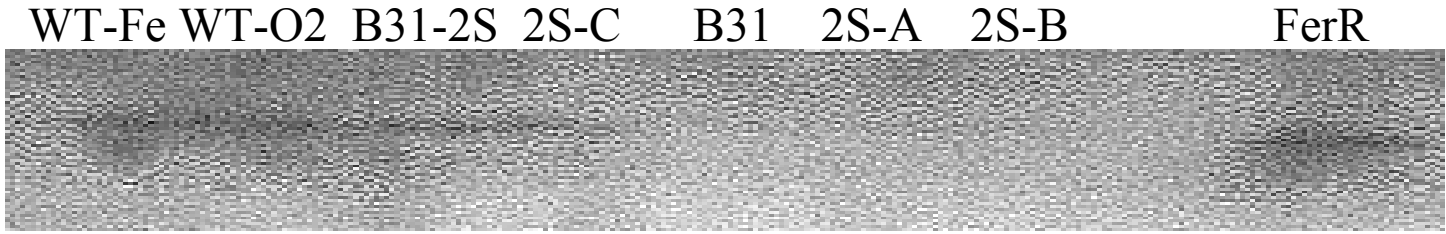
- 15,000 mutant colonies screened
- Identified 72 that were Fe(III) respiration-deficient
- 57 multiple deficiencies, 15 unique deficiency

Does not respire solid Fe(III) or solid Mn(III/IV), yet respires 15 other electron acceptors [including soluble Fe(III) and soluble Mn(III)]

B31 contains a mutation in GspE, the traffic ATPase of Type II protein secretion

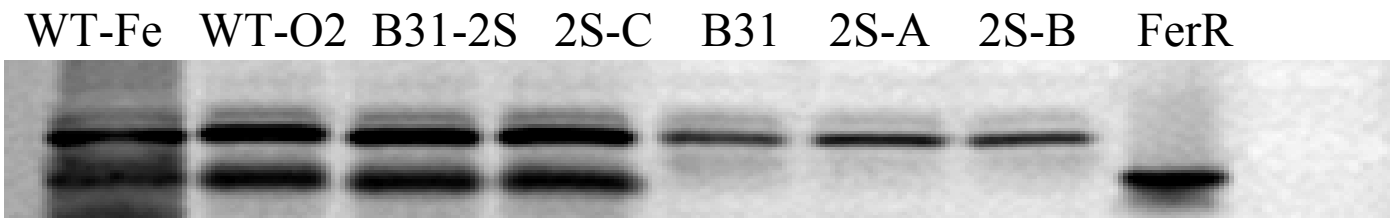


KCl wash to detach peripheral proteins from cell surface of WT and *gspE*



Native-PAGE
Ferrozine stain

- **Fe(III) reductase is missing from periphery of *gspE* mutant B31**



SDS-PAGE
Heme stain

MALDI-TOF MS/MS analysis of excised 91 kDa heme-positive protein:

QYLIDETSDLTPAVK

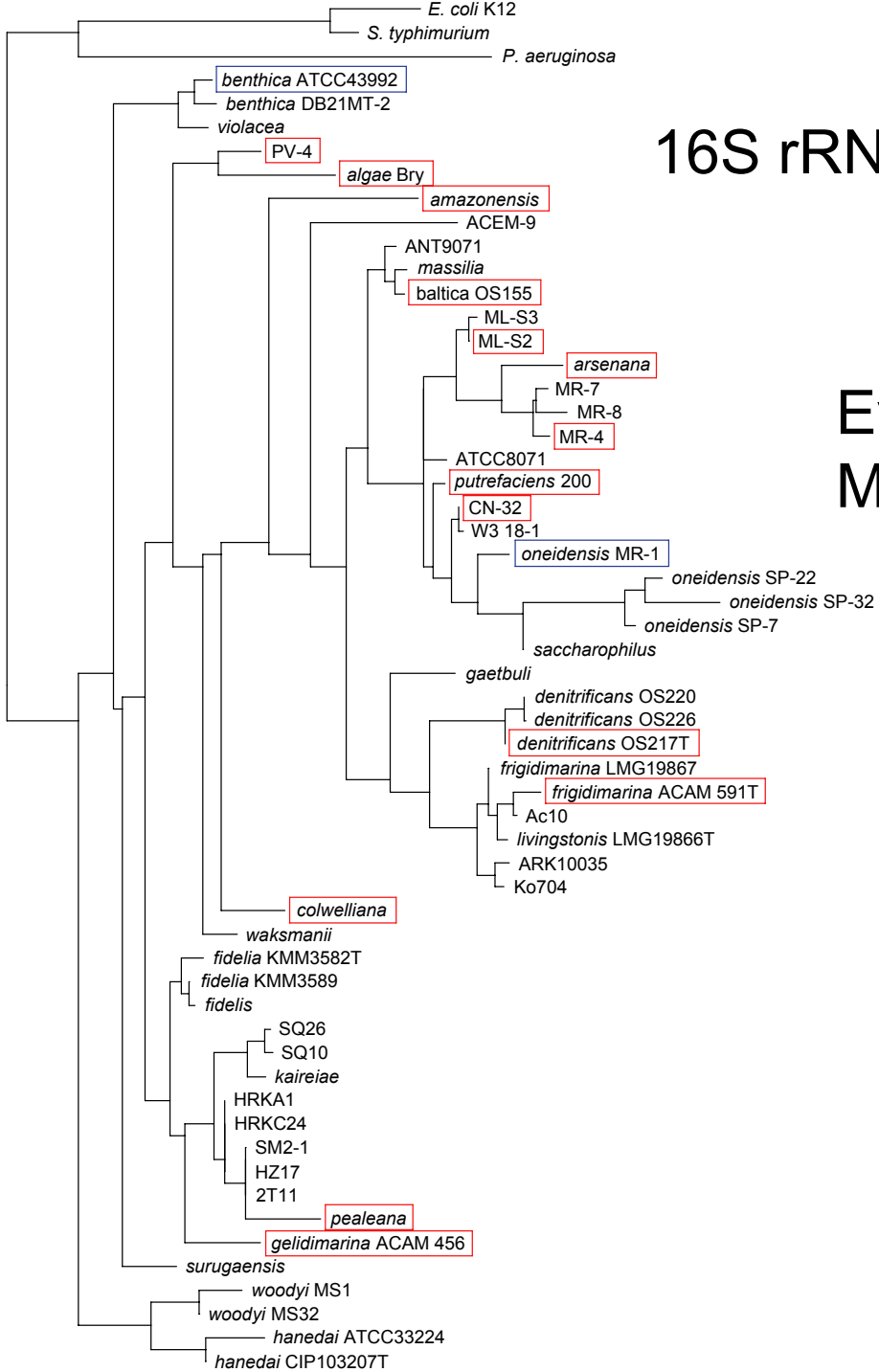
- **until January 2006:**

"15 amino acid sequence obtained from MALDI MS/MS did <u>not</u> display homology to any predicted protein-encoding ORF in the sequence databases, including the *S. oneidensis* MR-1 genome"

February 2006: MALDI-TOF MS/MS Identification of the *c*-type cytochrome missing from B31 outer membrane

Protein	Species	sequence homology to: QYLIDETSDLTPAVK	% Identity	% Similarity	e-value
MtrC	*Shewanella* sp. W3-18-1	QYLIDETCDLTPAVK (14/15)	100%	100%	0.0
MtrC	*S. putrefaciens* CN-32	QYLIDETCDLTPAVK (14/15)	78%	86%	0.0
MtrC	*Shewanella baltica* OS155	QYFGDETCDVAAT- (7/15)	77%	84%	0.0
MtrC	*Shewanella* sp. ANA-3	QYFGDETCDVAST-- (7/15)	75%	83%	0.0
MtrC	*Shewanella* sp. MR-7	QYFGDETCDVAST-- (7/15)	75%	83%	0.0
MtrC	*Shewanella* amazonensis	EYLLDETCDLPAA-- (9/15)	65%	78%	0.0
MtrC	*Shewanella* sp. MR-4	QGL---------- (2/15)	60%	71%	0.0
MtrC	*Shewanella frigidimarina* - 1	QFIGSEKA------- (2/15)	51%	64%	0.0
MtrC	*Shewanella frigidimarina* - 2	QFIGSEKA------ (2/15)	51%	65%	0.0
MtrC	*Shewanella* sp. PV-4	QYIGSQKA------ (2/15)	45%	59%	2e-151
MtrC	*Shewanella oneidensis* MR-1	QGL---------- (2/15)	48%	59%	9e-141
MtrF	*Shewanella oneidensis* MR-1	QHFTSETC-----AA (4/15)	29%	48%	2e-71
MtrF	*Shewanella* sp. PV-4	QYFTSESCN----D (4/15)	30%	44%	4e-70
MtrF	*Shewanella* sp. ANA-3	QHFTSETC-----AA (4/15)	28%	45%	3e-69
MtrF	*Shewanella* sp. MR-7	QHFTSETC-----AA (4/15)	28%	44%	7e-69
MtrF	*Shewanella* sp. MR-4	QHFTSETC-----AA (4/15)	28%	45%	4e-68
MtrF	*Shewanella amazonensis*	QYFGSENCAAT--- (5/15)	30%	44%	2e-65

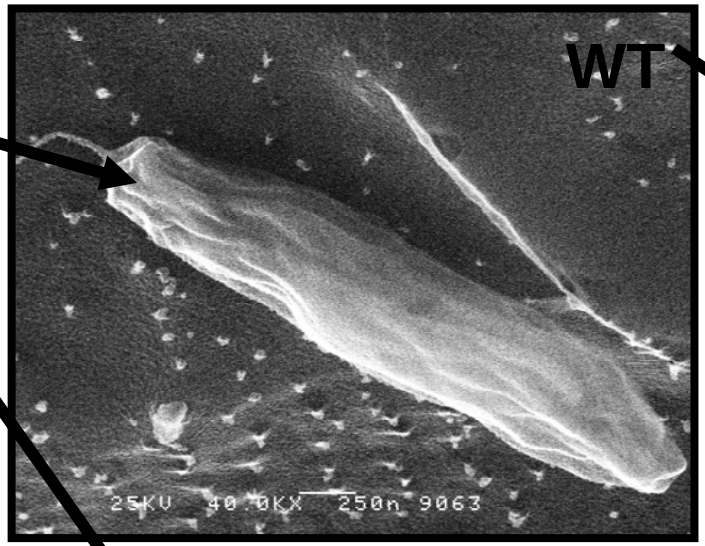
16S rRNA tree - *Shewanella* genus

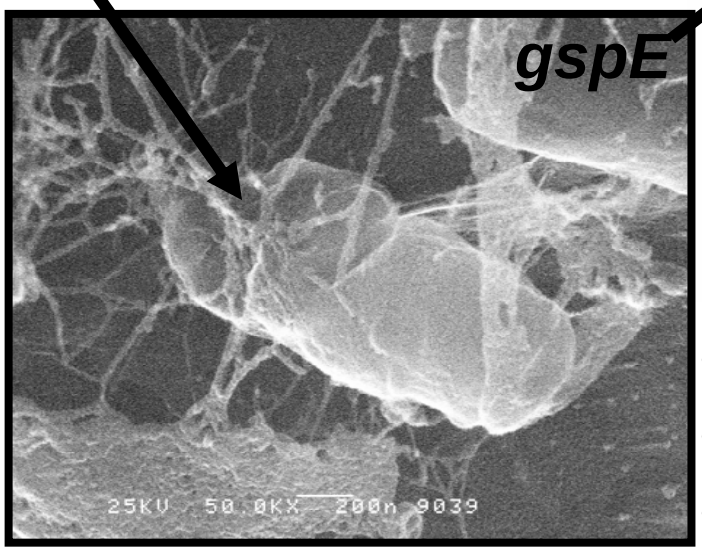


Evolutionary history of MtrC of MR-1?

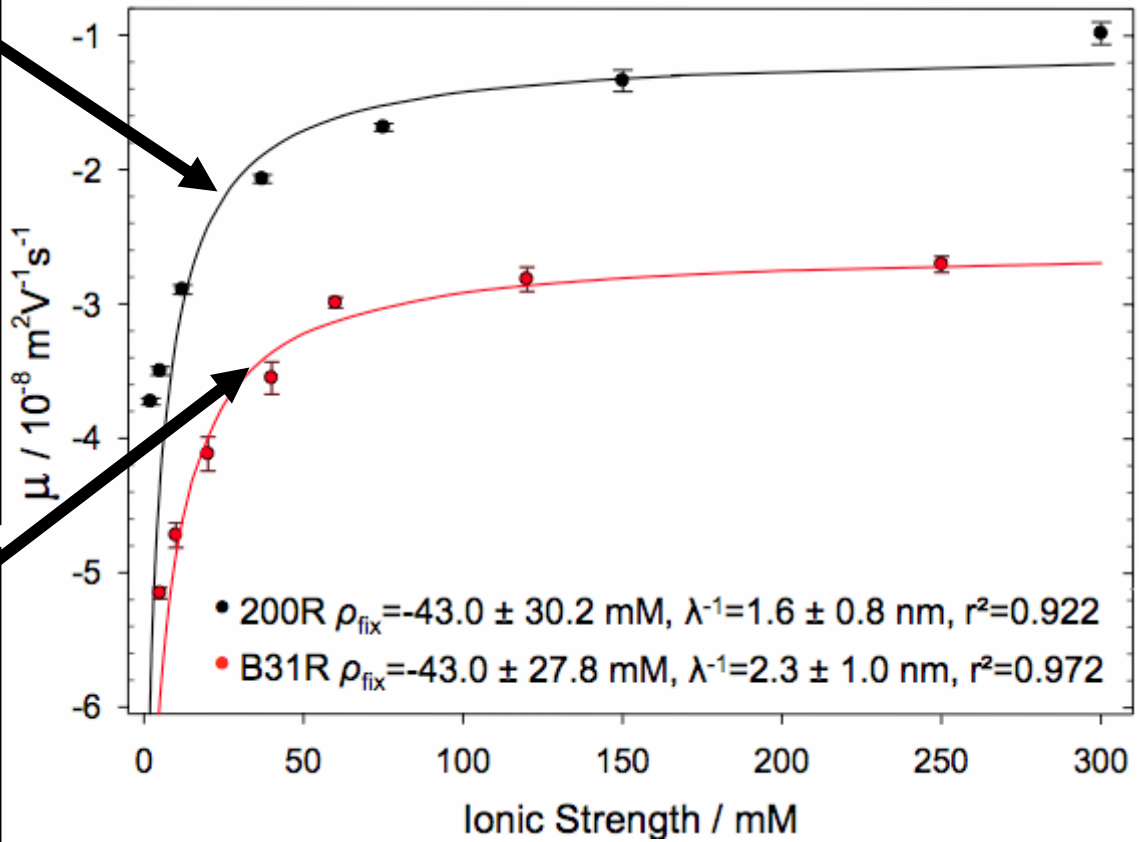
S. putrefaciens 200 WT versus Type II secretion mutant *gspE* (in collaboration with Andy Neal at SREL)

Cryo HR-SEM



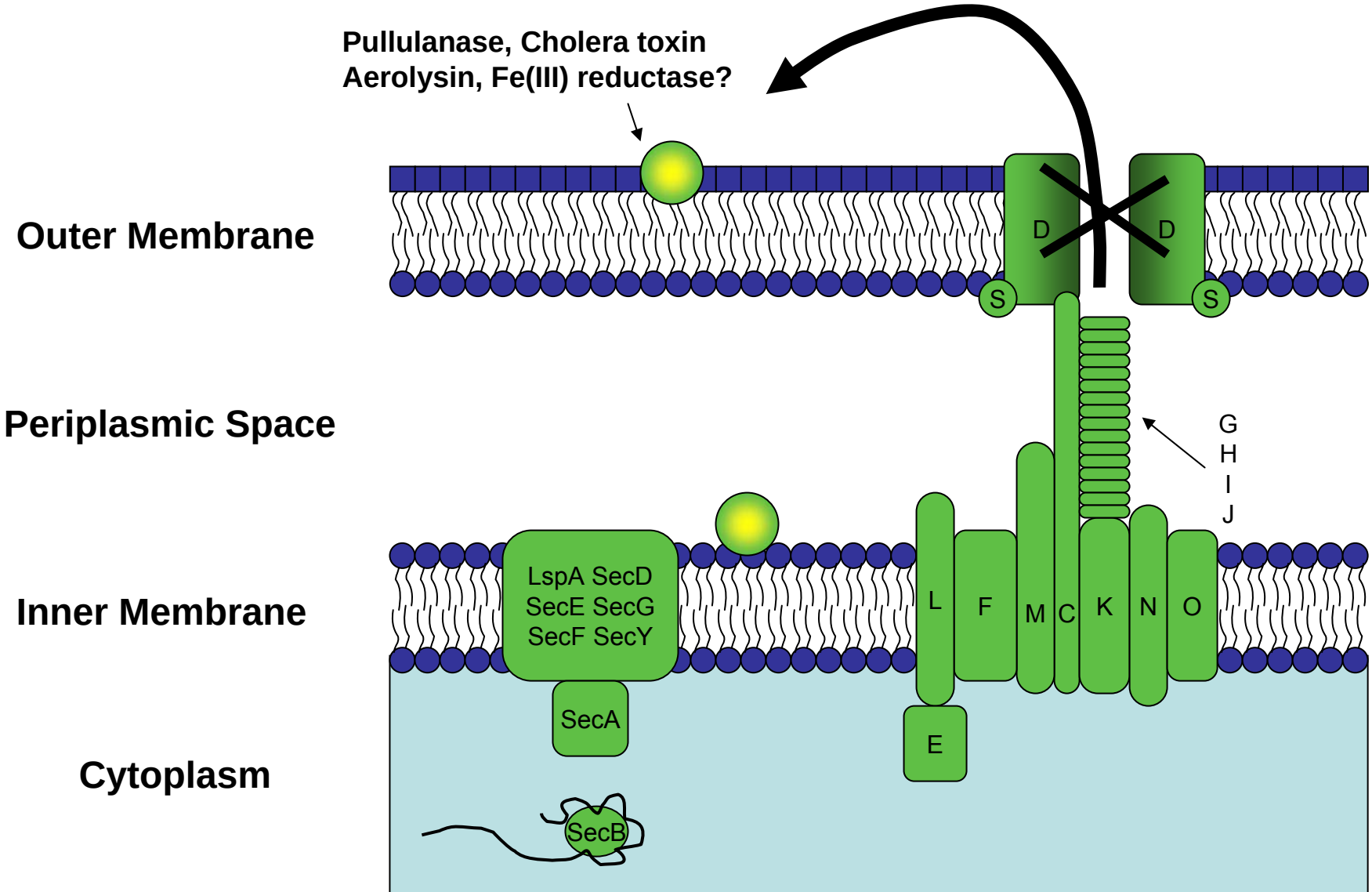


Electrophoretic mobility

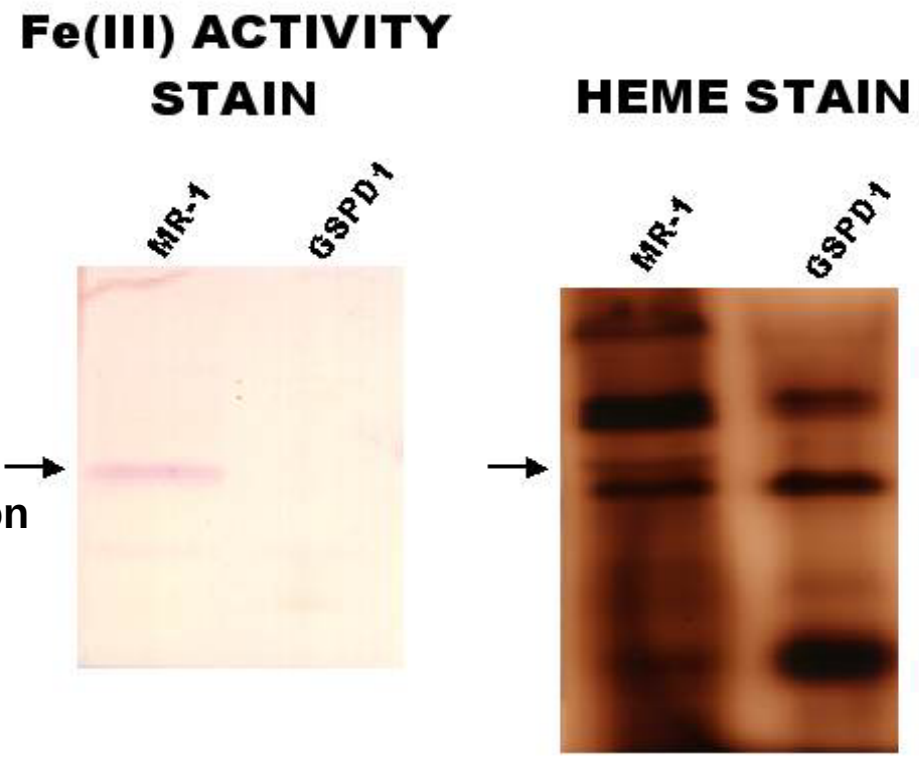


- Surface charge densities are similar
- GspE: thicker ion permeable layer (soft)
- Striking difference in surface features

Site-targeted deletion of the MR-1 Type II secretin GspD



Fe(III) reductase missing from peripheral proteins detached from outside face of *S. oneidensis* MR-1 outer membrane

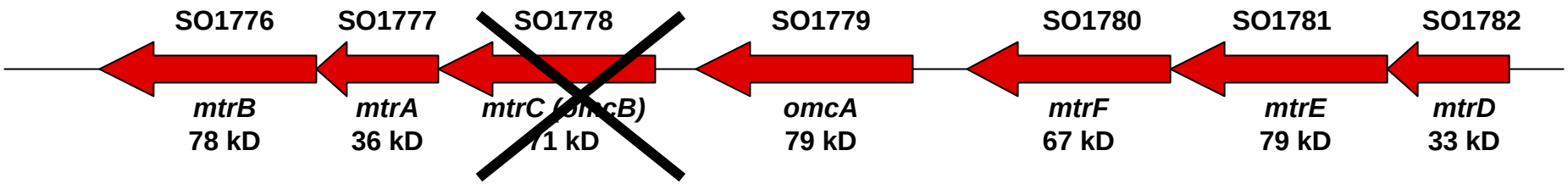


Fe(III) reductase complex missing in type II secretion mutant GspD1

Fe(III) reductase complex stains heme-positive

MALDI-TOF MS analysis of heme-positive protein in Fe(III) reductase of *S. oneidensis* MR-1: MtrC

Mtr region of *S. oneidensis* MR-1 genome

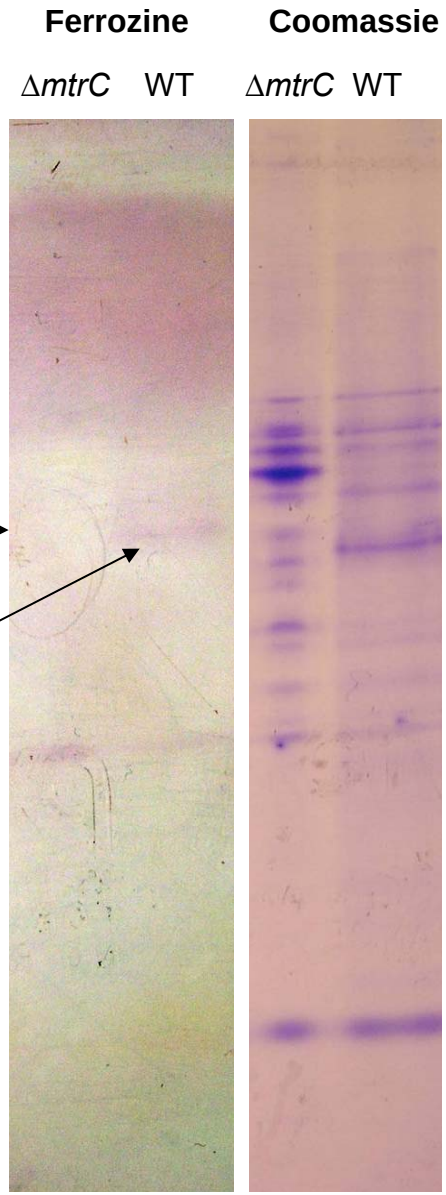


MtrB: putative outer membrane anchor (MtrE = second copy)
MtrA: periplasmic decaheme *c*-type cytochrome (MtrD = second copy)
MtrC: outer membrane decaheme *c*-type cytochrome (MtrF = second copy)
OmcA: outer membrane decaheme *c*-type cytochrome (no second copy)

MtrC deletion mutant does <u>not</u> respire on solid Fe(III),

and the activity of its Fe(III) reductase complex……..

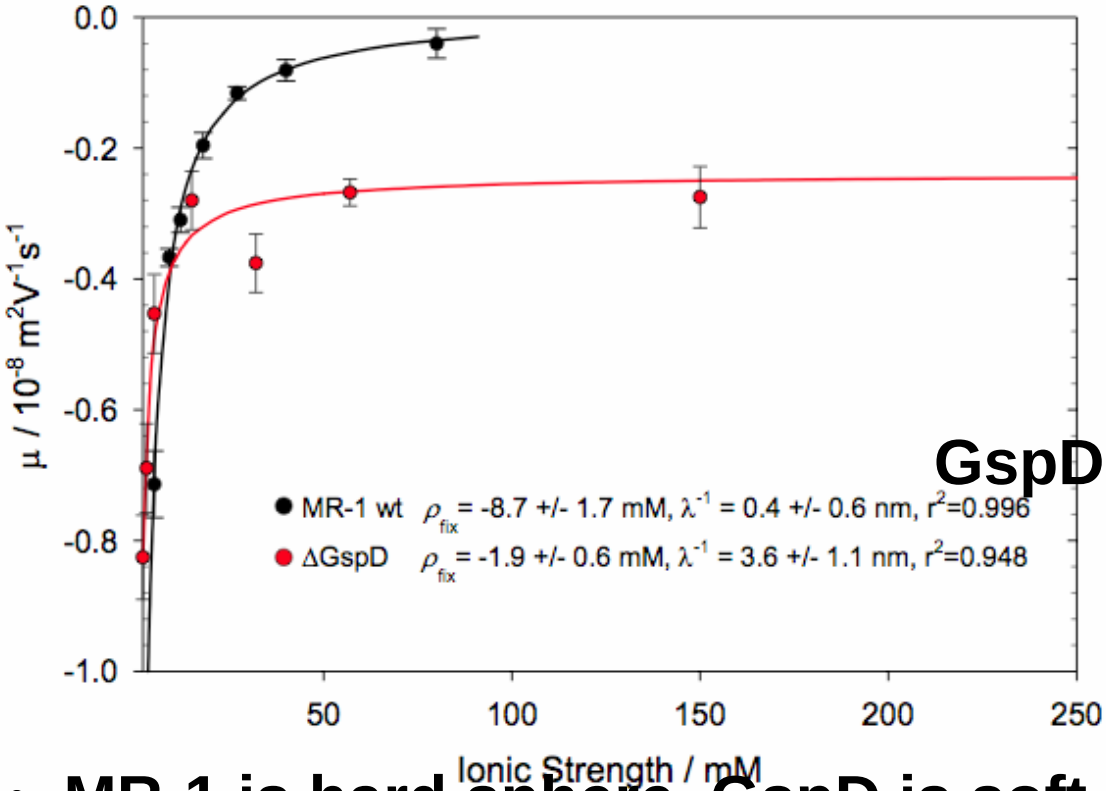
……..is severely impaired (Native PAGE Analysis of Δ*mtrC)*



MtrC is required for Fe(III) reductase activity, but not for formation of Fe(III) reductase protein complex

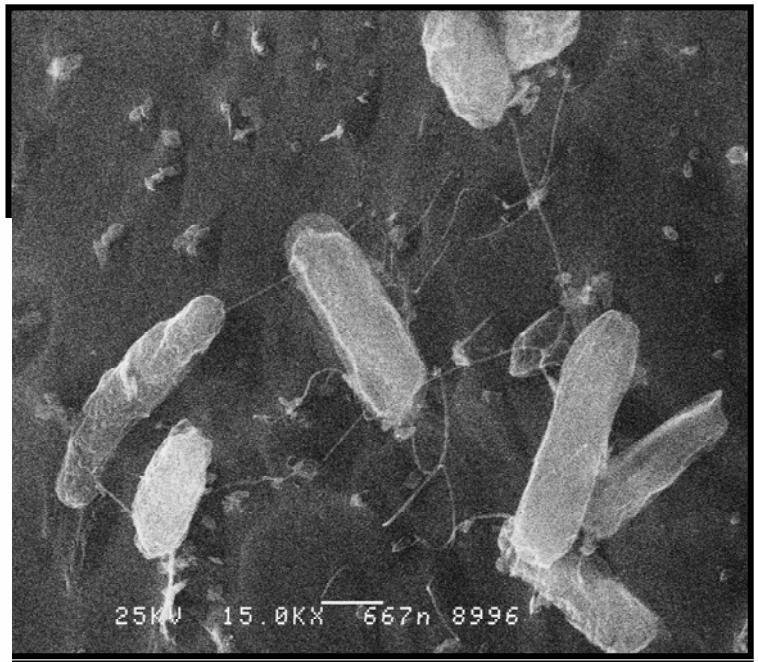
S. oneidensis MR-1 WT versus Type II secretion mutant *gspD*

Electrophoretic mobility:

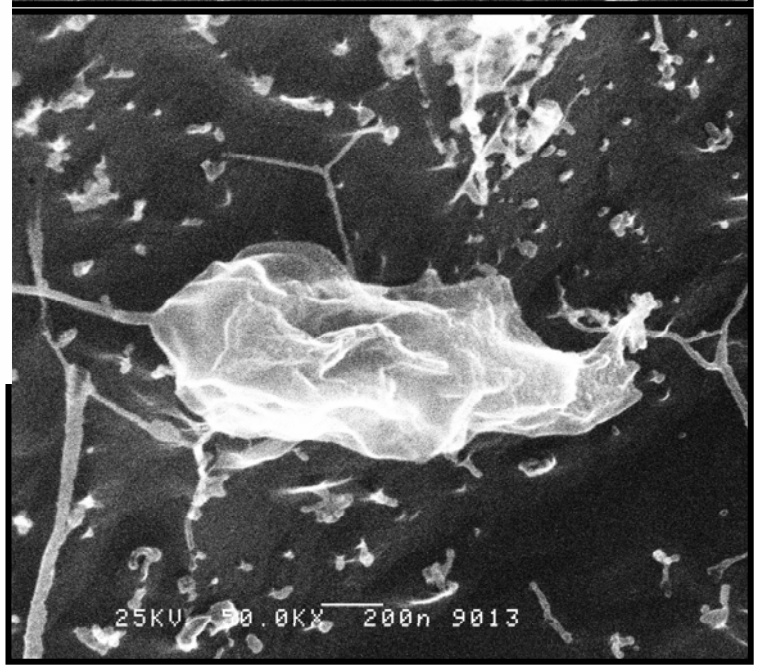


Cryo HR-SEM:

MR-1

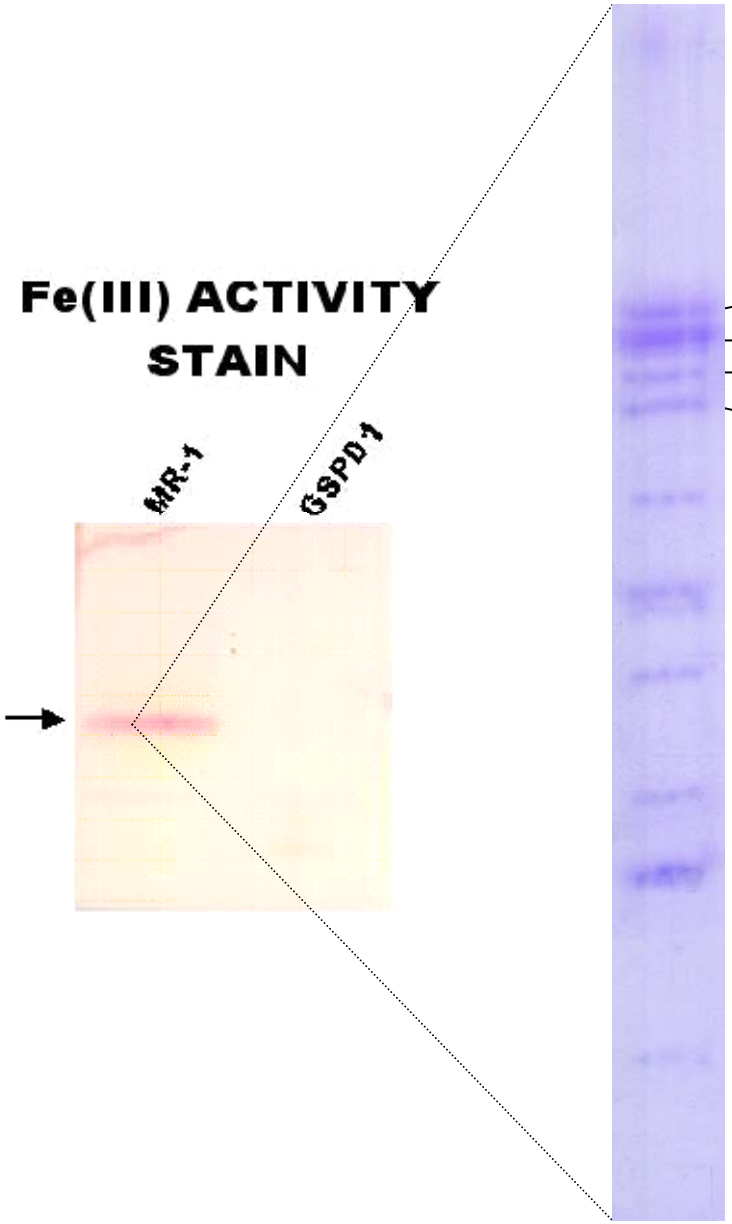


GspD



- MR-1 is hard sphere, GspD is soft
- MR-1 charge density is 5X higher
- GspD: ion permeable layer is 10X thicker and coated with EPS

MALDI-TOF MS identification of polypeptides in Fe(III) reductase complex of *S. oneidensis* MR-1



Autotransporter and one other protein

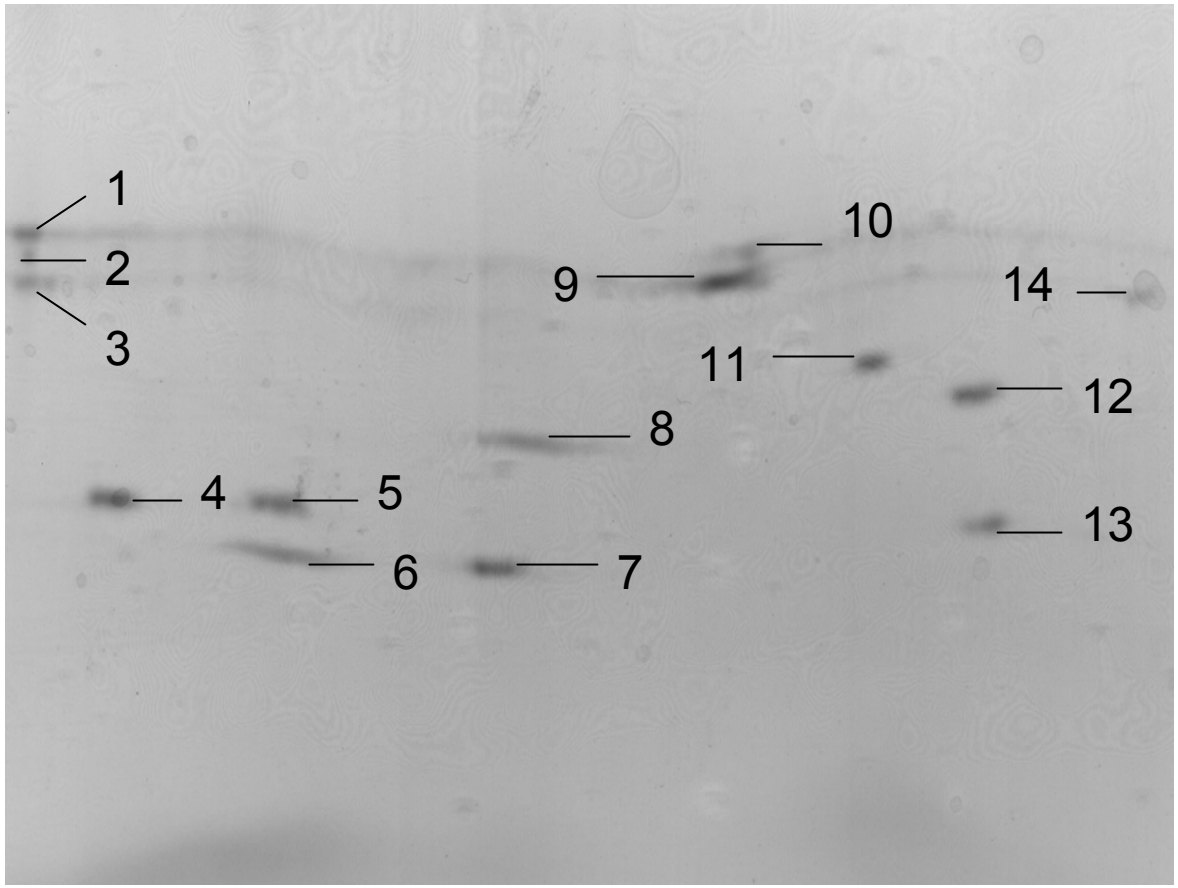
Autotransporter and and two other proteins

Autotransporter and two other proteins

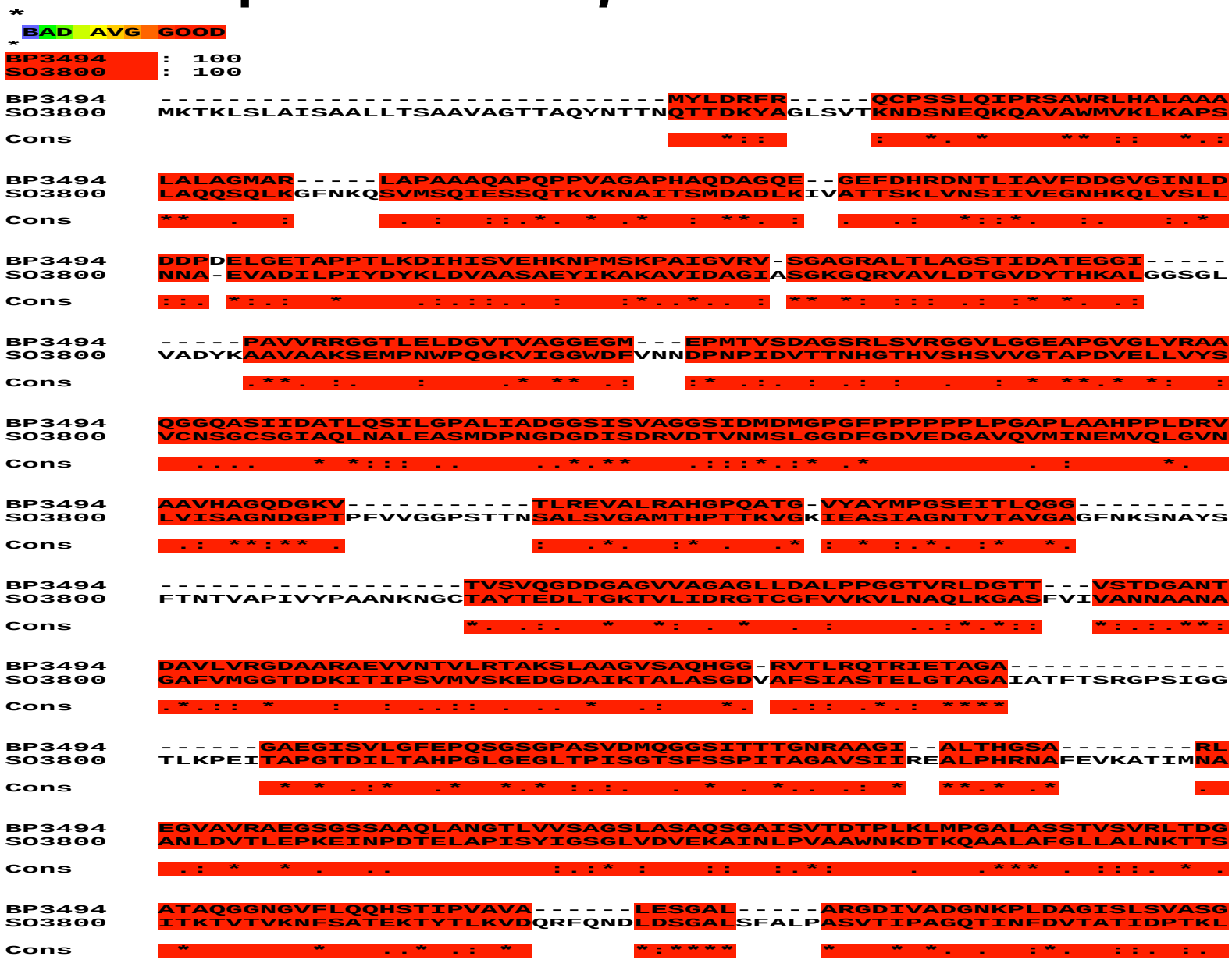
Autotransporter and *c*-type cytochrome MtrC

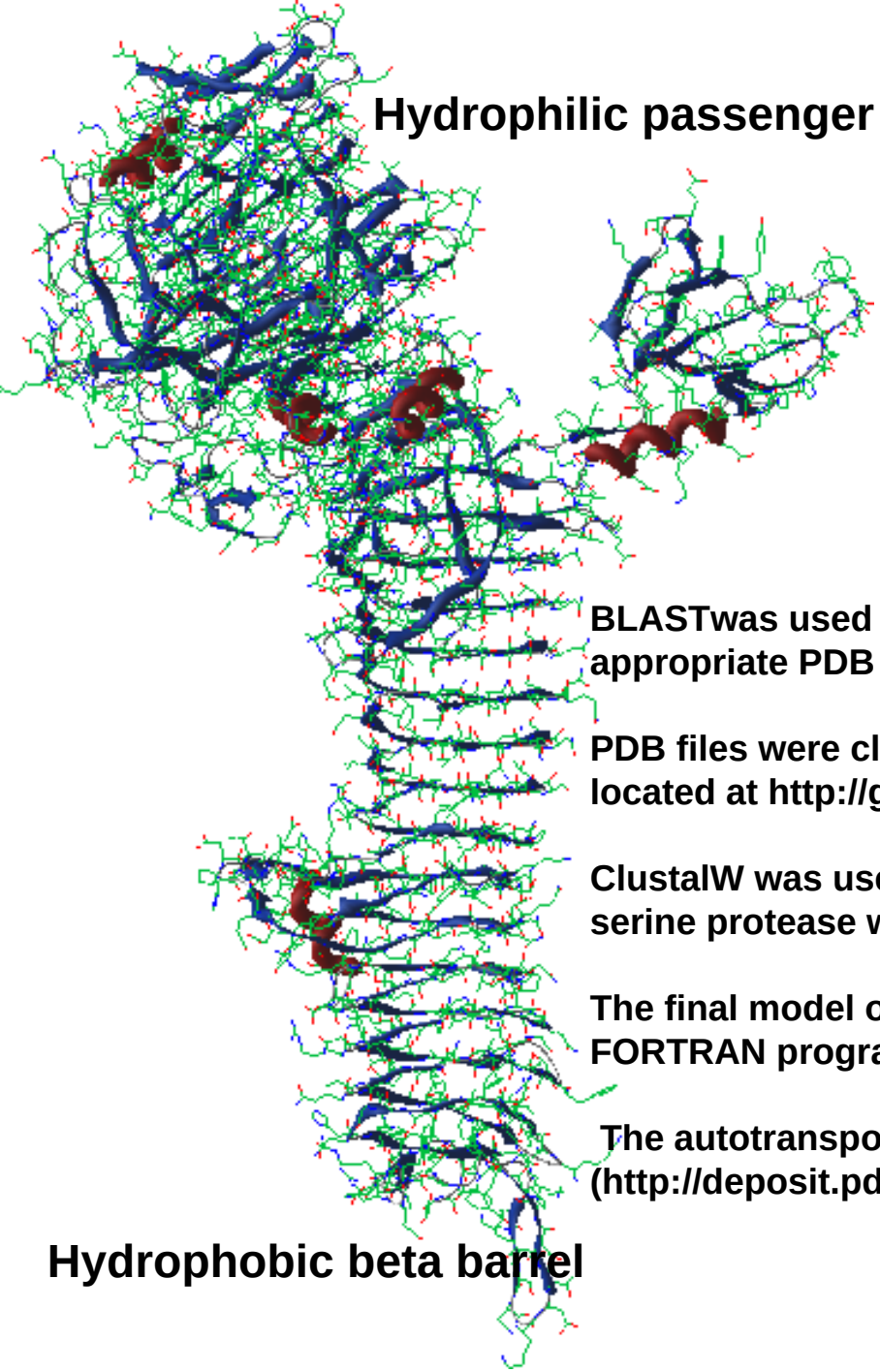
Currently being studied

2-D SDS PAGE of Fe(III) Reductase Complex



Secondary structure comparsion: autotransporters of *B. pertussis* and MR-1





Homology modeling of MR-1 autotransporter

BLASTwas used to search the protein databases for the most appropriate PDB structures to serve as templates for modeling.

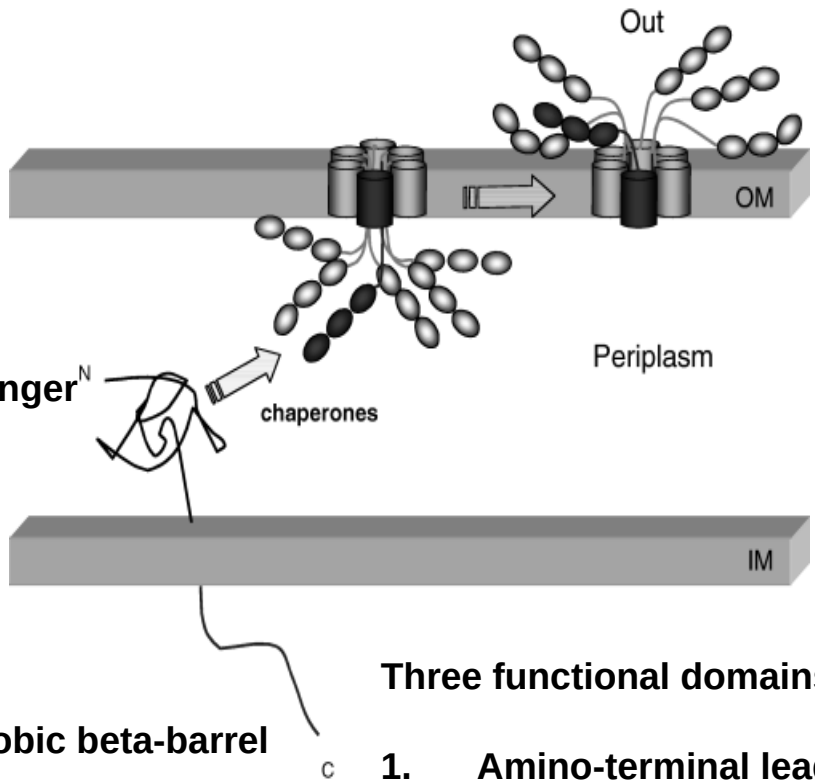
PDB files were cleaned-up using PDB Vacuum in the Modeler Toolbox, located at http://genomics.biology.gatech.edu/motool.

ClustalW was used to generate a multiple sequence alignment of the serine protease with templates that corresponded to conserved domains.

The final model of the autotransporter was generated with the FORTRAN program Modeller (http://salib.org/modeller/modeller/html).

The autotransporter homology model was validated with PROCHECK (http://deposit.pdb.org/cgi-bin/validate/adit-session-driver).

Autotransporter (Type V Secretion)



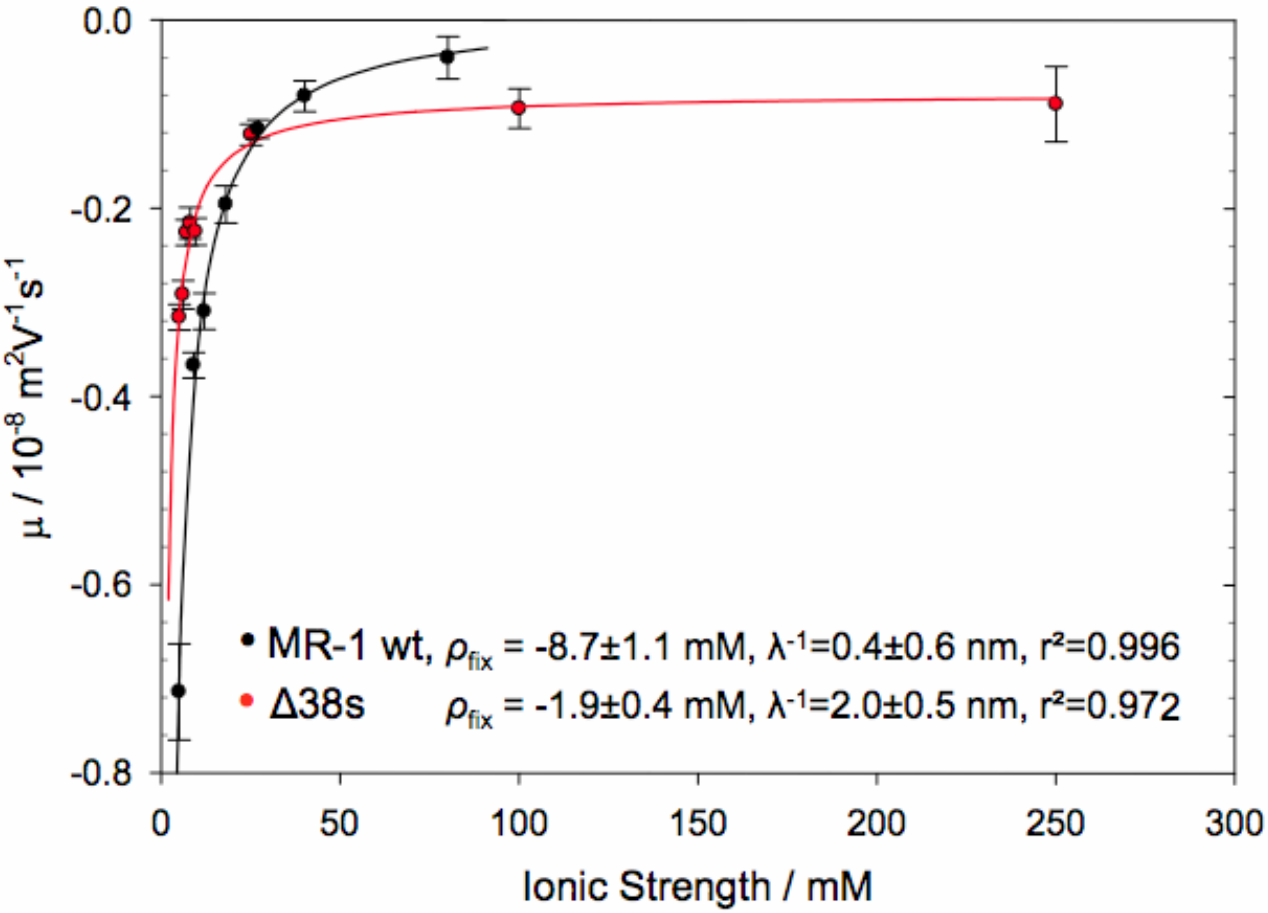
Hydrophilic passenger^{N}

Hydrophobic beta-barrel

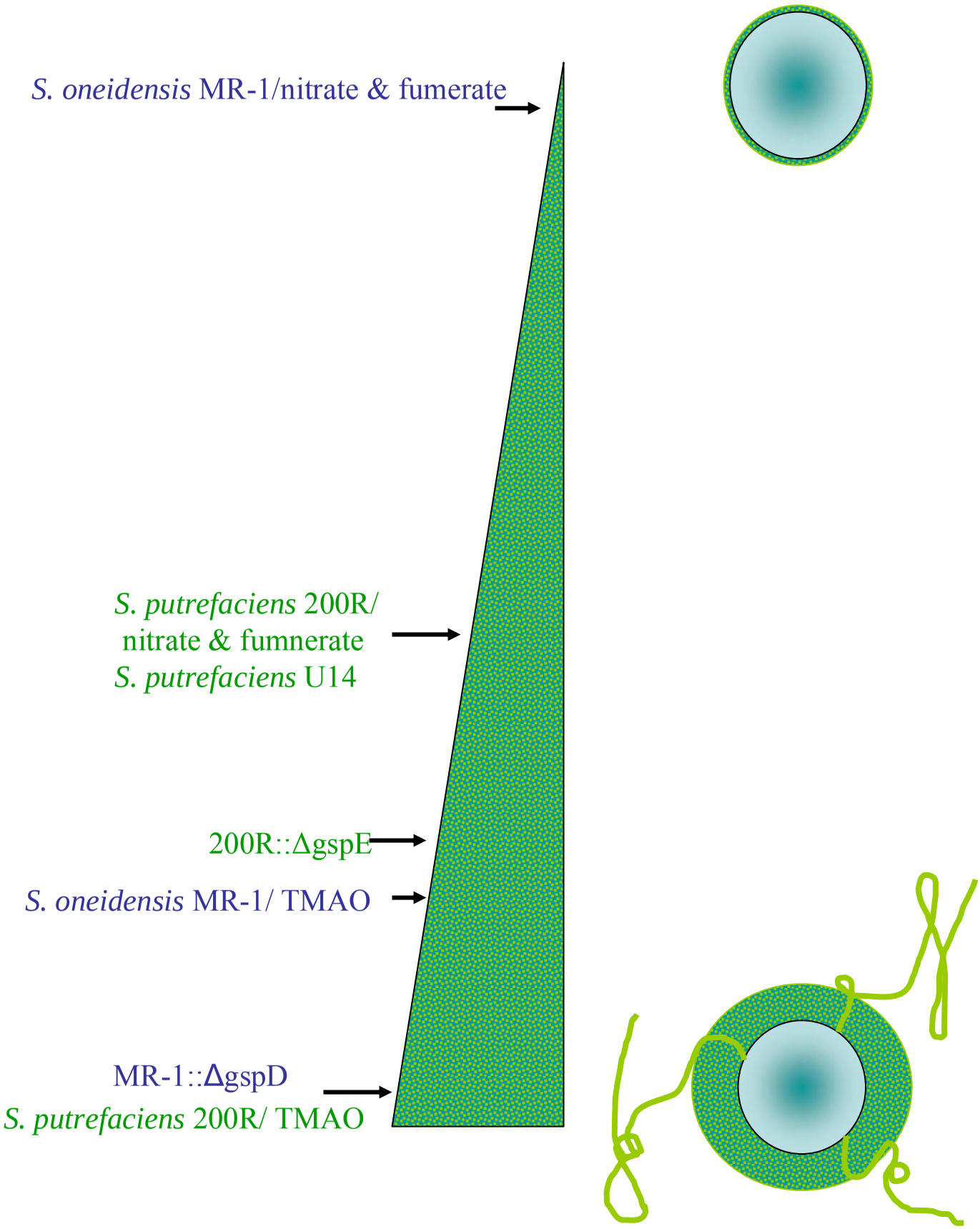
Three functional domains in autotransporters:

1. **Amino-terminal leader sequence**
2. **Amino-terminal passenger domain**
3. **Carboxy-terminal domain that forms a hexameric beta-barrel pore to allow secretion of passanger domain**

Electrophoretic mobility of autotransporter mutant



- **MR-1 is hard sphere, autotransporter mutant is 5X softer**
- **Autotransporter and GspD mutants are nearly identical in charge and softness**
- **Cryo HR-SEM not yet completed**


S. oneidensis MR-1/nitrate & fumerate
S. putrefaciens 200R/
nitrate & fumnerate
S. putrefaciens U14
200R::ΔgspE
S. oneidensis MR-1/ TMAO
MR-1::ΔgspD
S. putrefaciens 200R/ TMAO

Working Model